Spatial Characterization of Zinc in the 32-Cell Zebrafish (*Danio rerio*) Embryo Using X-ray Fluorescence Microprobe Imaging

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INTRODUCTION

Over the past 50 years, the importance of zinc to biological systems has gained an increasing amount of attention. Zinc has been shown to be essential for the function of a variety of enzymes from transcription factors to proteases [1]. Although zinc has also been demonstrated to be essential to the development, growth, and differentiation of all species, the role of zinc in embryogenesis is largely unknown.

Embryos from species which develop outside of the mature female presumably contain, at fertilization, all of the nutrients needed to develop into a viable organism. Recently, Vallee and colleagues working with *Xenopus laevis* (African Clawed Toad) oocytes [2] and Berg and colleagues working with *Danio rerio* (Zebrafish) embryos [3] have reported that a large amount of zinc (~1mM) is stored within the embryos at the time of fertilization. Subsequent studies using ^{65}Zn were performed on the *Xenopus* embryos to follow the distribution of zinc during embryonic development [4]. The results of these experiments showed that the zinc remained associated with the lipovitellin protein in yolk platelets until the organism reached the tadpole stage of development (~48 hours after fertilization). The interpretation of these results was that zinc did not play a role in the early stages of development but was stored in the lipoproteins until a much later stage of development, at which time it was distributed to other zinc metalloproteins as needed.

Due to the large concentration of zinc in the *Xenopus* and Zebrafish embryo, XANES measurements could be performed on intact single embryos to observe changes in the bulk zinc environment during development. The advantage of these measurements is that they give insight into zinc speciation without the necessity of chemical manipulation. Differences in the normalized XANES spectra clearly suggest that the bulk zinc environment *is* changing, even in the earliest stages of development [5]. The limitation of these results is that they address only the average zinc environment. X-ray fluorescence microprobe measurements provide the opportunity to gain more detailed information on the location of the zinc as well as insight into how the zinc environment is changes during the early stages of development.

RESULTS

Using a filtered white-beam of 2 μm x 2 μm area and 10 μm steps in both x- and y-directions, an image was made of the zinc fluorescence from a 32-cell Zebrafish embryo (Figure 1). The region of highest intensity zinc fluorescence corresponds to the yolk sac, the nutrient storage area of the embryo. More intriguing is the gradient of zinc fluorescence that appears at the boundary between the yolk sac and the animal cells and continues up into the animal cells. This gradient suggests that the zinc is migrating from the yolk sac into the animal cells which are actively dividing in ~15 minute intervals at the earliest stages of development. Images of the zinc fluorescence in embryos need to be made on a variety of embryos during the stages of meroblastic (incomplete) cleavage in order to establish an actual migration trend toward the animal cells.

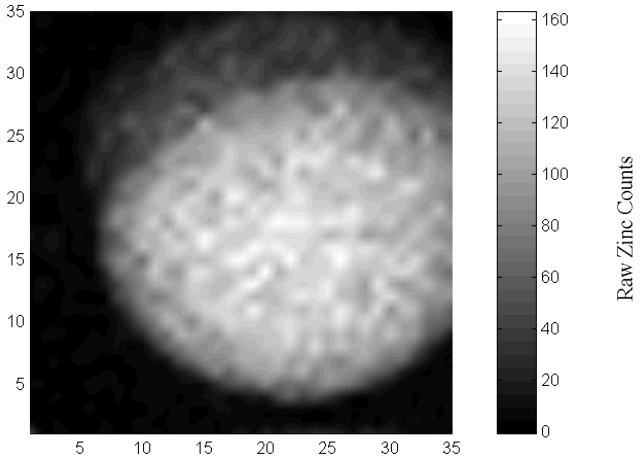

Figure 1. A gray-scale map of the zinc localization in the 32-cell Zebrafish embryo. The zinc is highly localized within the yolk sac. However, the substantial amount of zinc fluorescence in the region of the embryo where cytoplasmic bridges connect the animal cells and the yolk sac suggests there may be migration of zinc from the yolk sac to the rapidly dividing animal cells.

Although the zinc is largely localized in the yolk sac, the image demonstrates a significant concentration in the animal cells. This raises the question of why the zinc is present in the animal cells this early (1.75 hours after fertilization), if it is not utilized until much later in embryogenesis? Since the x-ray microprobe offers very small spatial resolution, the beam could be positioned in relatively intense areas of zinc fluorescence in both the animal and yolk regions of the embryo for microXANES measurements. The preliminary XANES spectra (Figure 2) show distinct differences, but they are difficult to characterize due to the noise level of the data. However, due to the sensitivity of XANES to changes in geometry as well as ligation, the fact that changes are observed in these XANES spectra implies that the zinc does not just exist as a stored nutrient for use in the latter stages of development but may play an integral role at even the earliest cleavage stages. Future efforts will be placed on improving the signal to noise ratio of the XANES measurements so that the changes may be characterized through comparisons with zinc model complexes and the purified embryo proteins. In determining the location as well as the coordination environment of the various zinc species present in the embryo, we can gain insight into the role(s) of zinc in embryogenesis.

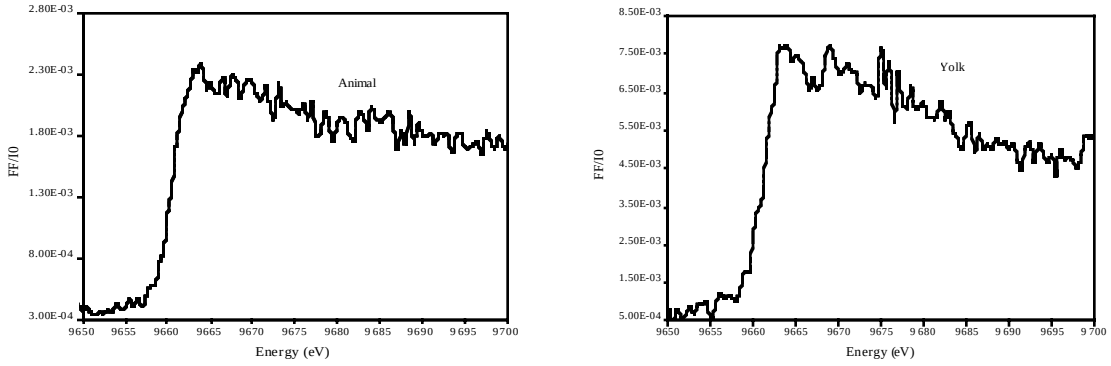

Figure 2. Preliminary microXANES measurements from the animal and yolk regions in the 32-cell Zebrafish embryo show significant position-dependent changes.

REFERENCES

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